

Serial No. 10/780,297
Filed: February 17, 2004

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AMENDMENTS TO THE CLAIMS

1. (Currently amended) A liquid pharmaceutical composition in the form of an aqueous solution comprising an amount of a human erythropoietin glycoprotein product [such as to provide] providing from about 10 µg to about 10,000 µg of erythropoietin protein per mL of solution, from about 10 to about 200 mmol per liter of said solution of a multiple charged inorganic anion selected from a sulfate[,] or phosphate [or citrate] anion, an antioxidant, and a pharmaceutically acceptable buffer, said anion and said buffer being present in said solution in an amount [such as to provide] providing the solution with a pH of from about 5.5 to about 7.0.
2. (Original) The composition of claim 1 wherein said solution is an isotonic solution.
3. (Canceled)
4. (Currently amended) The composition of claim 1 wherein the anion is [selected from the group consisting of sulfate or] a phosphate ion.
5. (Original) The composition of claim 4 wherein the anion is a sulfate anion.
6. (Currently amended) The composition of claim 1 wherein the pH is 5.8 to 6.7.
7. (Currently amended) The composition of claim 6 wherein the pH is 6.0 to 6.5.
8. (Original) The composition of claim 7 wherein the pH is about 6.2.
9. (Previously presented) The composition of claim 1 wherein the buffer is a phosphate buffer.

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10. (Original) The composition of claim 9 wherein the buffer is a phosphate buffer in an amount of from 10 to 50 mmol per liter of said solution.
11. (Canceled)
12. (Previously presented) The composition of claim 1 wherein the erythropoietin has the amino acid sequence of SEQ ID NO: 1 or SEQ ID NO: 2.
13. (Previously presented) The composition of claim 1 wherein the erythropoietin has the amino acid sequence SEQ ID NO: 1 or SEQ ID NO: 2 or having said sequence modified by the addition of from 1 to 6 glycosylation sites.
14. (Previously presented) The composition of claim 13 wherein the sequence modification is
- Asn³⁰Thr³²Val⁸⁷Asn⁸⁸Thr⁹⁰
15. (Previously presented) The composition of claim 13, wherein the erythropoietin has the sequence of human erythropoietin modified by a rearrangement of at least one glycosylation site.
16. (Original) The composition of claim 15, wherein the rearrangement comprises deletion of any of the N-linked glycosylation sites in human erythropoietin sequence with the addition of an N-linked glycosylation site at position 88 of the sequence of human erythropoietin.
17. (Canceled)
18. (Original) The composition of claim 1, wherein the glycoprotein product is a pegylated erythropoietin.
- 19-21. (Cancelled)

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22. (Previously presented) The liquid pharmaceutical composition of claim 5 wherein the glycoprotein product is a pegylated erythropoietin, and the buffer is present in an amount from 10 to 50 mmol per liter of said solution.

23-28. (Canceled)

29. (Previously presented) The composition of claim 22 wherein the pH is about 6.2.

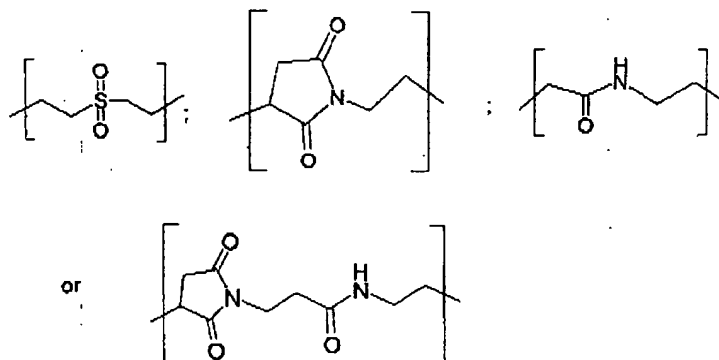
30. (Previously presented) The composition of claim 29 wherein the buffer is a phosphate buffer.

31-39. (Canceled)

40. (Previously presented) The composition of claim 18, wherein the pegylated erythropoietin glycoprotein product is a conjugate of an erythropoietin glycoprotein having at least one free amino group, said glycoprotein having the sequence SEQ ID NO: 1 or SEQ ID NO: 2 or having said sequence modified by the addition of from 1 to 6 glycosylation sites; said glycoprotein being covalently linked to from one to three lower-alkoxy poly(ethylene glycol) groups with each poly(ethylene glycol) group being covalently linked to the glycoprotein via a linker of the formula -C(O)-X-S-Y- with the C(O) of the linker forming an amide bond with one of said amino groups, X is $-(CH_2)_k-$ or $-CH_2(O-CH_2-CH_2)_k-$; and k is from 1 to 10; Y is selected from

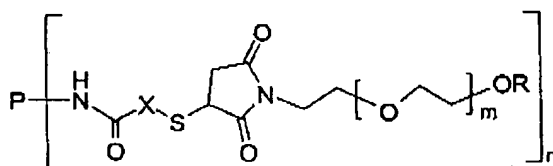
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the average molecular weight of each poly(ethylene glycol) moiety being from about 20 kilodaltons to about 40 kilodaltons; and the molecular weight of the conjugate being from about 51 kilodaltons to about 175 kilodaltons.

41. (Previously presented) The composition of claim 40 wherein said conjugate has the formula:

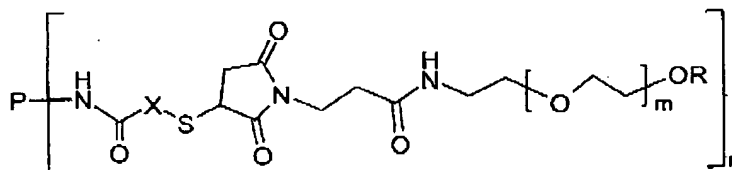


wherein n is an integer from 1 to 3; m is an integer from about 450 to about 900; R is lower alkyl; X is $-(\text{CH}_2)_k-$ or $-\text{CH}_2(\text{O}-\text{CH}_2-\text{CH}_2)_k-$, and P is the erythropoietin glycoprotein minus the amino group or groups which form an amide linkage with X and k is from 1 to 10.

42. (Previously presented) The composition of claim 41 wherein said conjugate has the formula:

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n is an integer from 1 to 3; m is an integer from about 450 to about 900; R is lower alkyl; X is $-(CH_2)_k-$ or $-CH_2(O-CH_2-CH_2)_k-$, and P is the erythropoietin glycoprotein minus the amino group or groups which form an amide linkage; and k is from 1 to 10.

43. (Previously presented) The composition of claim 1 wherein said solution contains from 100 μ g to 400 μ g erythropoietin protein per ml of solution, from 10 to 200 mmol/liter of solution of a sulfate as the multiple charged inorganic anion, and 10 to 50 mmol/liter of solution of a phosphate as the pharmaceutically acceptable buffer, said solution having a pH of from about 6.0 to about 6.5.

44. (Previously presented) The composition of claim 43 wherein the antioxidant is methionine which is present in an amount up to 20 mM said composition further comprising 1 - 5 % of a polyol (w/v).

45. (Currently amended) The composition of claim 44 consisting essentially of [10] 100 μ g to [10000] 400 μ g erythropoietin protein per ml of solution, 40 mmol/liter of solution of the sulfate, 10 mmol/liter of said solution of the phosphate, 10 mM methionine, said composition having a pH of about 6.2, and wherein the polyol is mannitol which is present in the solution at 3% (w/v).

46. (Previously presented) The composition of claim 1 wherein the solution contains 10 μ g to 10000 μ g erythropoietin protein per ml of solution, the buffer is phosphate which is present at 10 to 50 mmol/liter of solution, said solution further containing NaCl which is present at 10 to 100 mmol/liter of solution and having a pH of from about 6.0 to about 7.0.

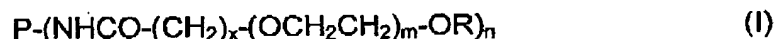
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47. (Previously presented) The composition of claim 46 wherein the NaCl is present at 100 mmol/liter of solution, the phosphate is present at 10 mmol/l, said solution containing 10 mM methionine and having a pH of about 7.0.

48. (Previously presented) The composition of claim 1 wherein the amount of erythropoietin protein is 50, 100, 400, 800 or 2,500 µg/ml of solution.

49. (Currently amended) A liquid pharmaceutical composition in the form of an aqueous solution comprising a therapeutically effective amount of a pegylated erythropoietin glycoprotein product of formula



wherein

P is an erythropoietin glycoprotein having the sequence SEQ ID NO: 1, SEQ ID NO: 2, or either of these sequences modified by the addition of from 1 to 6 glycosylation sites or by rearrangement of at least one glycosylation site, minus the amino group of said glycoprotein which forms an amide linkage;

R is lower alkyl;

x is 2 or 3;

m is from about 450 to about 900;

n is from 1 to 3; and

wherein the values of n and m are such that the molecular weight of the conjugate minus the erythropoietin glycoprotein is from 20 kilodaltons to 100 kilodaltons;

an [antioxidant] antioxidant, from about 10 to about 200 mmol/L of a multiple charged inorganic anion selected from a sulfate[,], or phosphate [or citrate] anion; and a pharmaceutically acceptable buffer, said anion and said buffer being present in said solution in an amount such that the pH of the solution is from about 5.5 to about 7.0.

50. (Original) The composition of claim 49 wherein x is 2, m is 650 to 750, n is 1, and R is methyl.

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51. (Previously presented) The liquid pharmaceutical composition of claim 49 wherein the pegylated erythropoietin glycoprotein product is present in an amount of from about 10 μg to about 10,000 μg per ml of said liquid composition, and the pharmaceutically acceptable buffer is present in an amount of from about 10 to about 50 mmol per liter of said liquid composition.

52. (Original) The composition of claim 51 wherein x is 2, m is 650 to 750, n is 1, and R is methyl.

53. (Previously presented) The liquid pharmaceutical composition of claim 50 wherein the pegylated erythropoietin glycoprotein is present in an amount of about 100.0 $\mu\text{g/mL}$ of solution, the multiple charged inorganic anion is sodium sulfate which is present in an amount of about 5.68 mg/mL, the pharmaceutically acceptable buffer is sodium phosphate which is present in an amount of about 1.38 mg/mL, and wherein the pH of the solution is about 6.2 ± 0.2 .

54. (Previously presented) The liquid pharmaceutical composition of claim 53 wherein the antioxidant is methionine which is present in an amount of about 1.49 mg/mL, said composition further comprising mannitol in an amount of about 30.0 mg/mL and poloxamers type 188 in an amount of 0.1 mg/mL.

55. (Previously presented) The liquid pharmaceutical composition of claim 50 wherein the pegylated erythropoietin glycoprotein is present in an amount of about 400 $\mu\text{g/mL}$ of solution, the multiple charged inorganic anion is sodium sulfate which is present in an amount of about 5.68 mg/mL, the pharmaceutically acceptable buffer is sodium phosphate which is present in an amount of about 1.38 mg/mL, and wherein the pH of the solution is about 6.2 ± 0.2 .

56. (Previously presented) The liquid pharmaceutical composition of claim 55 wherein the antioxidant is methionine which is present in an amount of about 1.49

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mg/mL, said composition further comprising mannitol in an amount of about 30.0 mg/mL and poloxamers type 188 in an amount of 0.1 mg/mL.

57. (Previously presented) The liquid pharmaceutical composition of claim 50 wherein the pegylated erythropoietin glycoprotein is present in an amount of about 800.0 µg/mL of solution, the multiple charged inorganic anion is sodium sulfate which is present in an amount of about 5.68 mg/mL, the pharmaceutically acceptable buffer is sodium phosphate which is present in an amount of about 1.38 mg/mL, and wherein the pH of the solution is about 6.2 ± 0.2 .

58. (Previously presented) The liquid pharmaceutical composition of claim 57 wherein the antioxidant is methionine which is present in an amount of about 1.49 mg/mL, said composition further containing mannitol in an amount of about 30.0 mg/mL and poloxamers type 188 in an amount of 0.1 mg/mL.

59. (Previously presented) The composition of claim 9 wherein the erythropoietin is present at about 25 to about 2,500 µg/ml, the buffer is sodium or potassium phosphate which is present in an amount of about 10 mM, said composition further containing NaCl which is present in an amount of about 100 mM and having a pH of about 7.0.

60. (Previously presented) The composition of claim 1 further comprising a non-ionic detergent in an amount up to 1% (w/v).

61. (Previously presented) The composition of claim 60 wherein the non-ionic detergent is present in an amount from about 0.001 (w/v) to about 0.01 (w/v).

62. (Previously presented) The composition of claim 61 wherein the non-ionic detergent is poloxamers type 188.

63. (Previously presented) The composition of claim 22 further comprising a non-ionic detergent in an amount up to 1% (w/v).

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64. (Previously presented) The composition of claim 63 wherein the non-ionic detergent is present in an amount from about 0.001 (w/v) to about 0.01 (w/v).
65. (Previously presented) The composition of claim 64 wherein the non-ionic detergent is poloxamers type 188.
66. (Previously presented) The composition of claim 49 further comprising a non-ionic detergent in an amount up to 1% (w/v).
67. (Previously presented) The composition of claim 66 wherein the non-ionic detergent is present in an amount from about 0.001 (w/v) to about 0.01 (w/v).
68. (Previously presented) The composition of claim 67 wherein the non-ionic detergent is poloxamers type 188.
69. (Previously presented) The composition of claim 1 wherein the antioxidant is methionine.
70. (Previously presented) The composition of claim 1 further comprising a polyol.
71. (Previously presented) The composition of claim 70 wherein the polyol is selected from the group consisting of mannitol, glycerol, trehalose and saccharose.
72. (Previously presented) The composition of claim 71 wherein the polyol is mannitol.
73. (Currently amended) The composition of claim 1 wherein the antioxidant is selected from the group consisting of cysteine, acetylcysteine or ascorbic acid.
74. (Previously presented) The composition of claim 49 wherein the antioxidant is methionine.

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75. (Previously presented) The composition of claim 74 wherein the methionine is present in a concentration of 1 to 20 mM.

76. (Previously presented) The composition of claim 49 wherein the inorganic anion is a sulfate anion.